```
=>  d his ful

     (FILE 'HOME' ENTERED AT 11:41:15 ON 04 MAY 2006)

     FILE 'REGISTRY' ENTERED AT 11:41:34 ON 04 MAY 2006
                E VITAMIN D 24/CN
L1            2 SEA ABB=ON  ("VITAMIN D 24-HYDROXYLASE (MOUSE PRECURSOR)"/CN
                OR "VITAMIN D 25-HYDROXYLASE"/CN)
                E CYP24/CN
                E CYP 24/CN

     FILE 'HCAPLUS' ENTERED AT 11:42:29 ON 04 MAY 2006
L2          302 SEA ABB=ON  L1 OR ?VITAMIN?(W)D(W)24(W)?HYDROXYLASE?
L3           26 SEA ABB=ON  L2 AND CYP24
L4           10 SEA ABB=ON  L3 AND MRNA
L5           26 SEA ABB=ON  L3 OR L4
L6            8 SEA ABB=ON  L5 AND ?PROTEIN?
L7           26 SEA ABB=ON  L5 OR L6
L8            9 SEA ABB=ON  L7 AND (?DETECT? OR ?IDENT? OR ?ISOLAT?)
L9           26 SEA ABB=ON  L7 OR L8
L10           8 SEA ABB=ON  L9 AND (PRD<19990402 OR PD<19990402) 8 cits from CA Plus

     FILE 'MEDLINE, BIOSIS, EMBASE, JAPIO, JICST-EPLUS' ENTERED AT 11:45:31 ON
     04 MAY 2006
L11         119 SEA ABB=ON  L9
L12          92 DUP REMOV L11 (27 DUPLICATES REMOVED)
L13          41 SEA ABB=ON  L12 AND (MRNA OR RECOMB?)
L14          21 SEA ABB=ON  L13 AND ?PROTEIN? 21 cits from d.b.'s

     FILE 'USPATFULL' ENTERED AT 11:50:23 ON 04 MAY 2006
L15           1 SEA ABB=ON  L9 AND (PRD<19990402 OR PD<19990402) 1 at from USPatFull

     FILE HOME

     FILE REGISTRY
     Property values tagged with IC are from the ZIC/VINITI data file
     provided by InfoChem.

     STRUCTURE FILE UPDATES:    3 MAY 2006  HIGHEST RN 882736-15-4
     DICTIONARY FILE UPDATES:   3 MAY 2006  HIGHEST RN 882736-15-4

     New CAS Information Use Policies, enter HELP USAGETERMS for details.

     TSCA INFORMATION NOW CURRENT THROUGH January 6, 2006

       Please note that search-term pricing does apply when
       conducting SmartSELECT searches.

     ************************************************************
     *                                                          *
     * The CA roles and document type information have been removed from *
     * the IDE default display format and the ED field has been added,   *
     * effective March 20, 2005.  A new display format, IDERL, is now    *
     * available and contains the CA role and document type information. *
     *                                                          *
     ************************************************************

     Structure search iteration limits have been increased.  See HELP SLIMITS
     for details.
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REGISTRY includes numerically searchable data for experimental and predicted properties as well as tags indicating availability of experimental property data in the original document. For information on property searching in REGISTRY, refer to:

http://www.cas.org/ONLINE/UG/regprops.html

FILE HCAPLUS

FILE COVERS 1907 - 4 May 2006 VOL 144 ISS 19
FILE LAST UPDATED: 3 May 2006 (20060503/ED)

New CAS Information Use Policies, enter HELP USAGETERMS for details.

This file contains CAS Registry Numbers for easy and accurate substance identification.

FILE MEDLINE
FILE LAST UPDATED: 3 MAY 2006 (20060503/UP). FILE COVERS 1950 TO DATE.

On December 11, 2005, the 2006 MeSH terms were loaded.

The MEDLINE reload for 2006 is now (26 Feb.) available. For details on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>). See also:

http://www.nlm.nih.gov/mesh/
http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

OLDMEDLINE is covered back to 1950.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2006 vocabulary.

This file contains CAS Registry Numbers for easy and accurate substance identification.

FILE BIOSIS
FILE COVERS 1969 TO DATE.
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 3 May 2006 (20060503/ED)

FILE EMBASE
FILE COVERS 1974 TO 4 May 2006 (20060504/ED)

EMBASE has been reloaded. Enter HELP RLOAD for details.

EMBASE is now updated daily. SDI frequency remains weekly (default) and biweekly.

This file contains CAS Registry Numbers for easy and accurate substance identification.

FILE JAPIO
FILE LAST UPDATED: 3 APR 2006 <20060403/UP>
FILE COVERS APRIL 1973 TO DECEMBER 22, 2005

>>> GRAPHIC IMAGES AVAILABLE <<<

>>> NEW IPC8 DATA AND FUNCTIONALITY NOT YET AVAILABLE IN THIS FILE. USE IPC7 FORMAT FOR SEARCHING THE IPC. WATCH THIS SPACE FOR FURTHER DEVELOPMENTS AND SEE OUR NEWS SECTION FOR FURTHER INFORMATION ABOUT THE IPC REFORM <<<

FILE JICST-EPLUS
FILE COVERS 1985 TO 1 MAY 2006 (20060501/ED)

THE JICST-EPLUS FILE HAS BEEN RELOADED TO REFLECT THE 1999 CONTROLLED TERM (/CT) THESAURUS RELOAD.

FILE USPATFULL
FILE COVERS 1971 TO PATENT PUBLICATION DATE: 4 May 2006 (20060504/PD)
FILE LAST UPDATED: 4 May 2006 (20060504/ED)
HIGHEST GRANTED PATENT NUMBER: US7039955
HIGHEST APPLICATION PUBLICATION NUMBER: US2006095999
CA INDEXING IS CURRENT THROUGH 2 May 2006 (20060502/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 4 May 2006 (20060504/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Feb 2006
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Feb 2006

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=> d que stat l10
L1            2 SEA FILE=REGISTRY ABB=ON  ("VITAMIN D 24-HYDROXYLASE (MOUSE
                PRECURSOR)"/CN OR "VITAMIN D 25-HYDROXYLASE"/CN)
L2          302 SEA FILE=HCAPLUS ABB=ON  L1 OR ?VITAMIN?(W)D(W)24(W)?HYDROXYLAS
                E?
L3           26 SEA FILE=HCAPLUS ABB=ON  L2 AND CYP24
L4           10 SEA FILE=HCAPLUS ABB=ON  L3 AND MRNA
L5           26 SEA FILE=HCAPLUS ABB=ON  L3 OR L4
L6            8 SEA FILE=HCAPLUS ABB=ON  L5 AND ?PROTEIN?
L7           26 SEA FILE=HCAPLUS ABB=ON  L5 OR L6
L8            9 SEA FILE=HCAPLUS ABB=ON  L7 AND (?DETECT? OR ?IDENT? OR
                ?ISOLAT?)
L9           26 SEA FILE=HCAPLUS ABB=ON  L7 OR L8
L10           8 SEA FILE=HCAPLUS ABB=ON  L9 AND (PRD<19990402 OR PD<19990402)

=> d ibib abs l10 1-8

L10  ANSWER 1 OF 8  HCAPLUS  COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER:         1999:762453  HCAPLUS
DOCUMENT NUMBER:          132:87714
TITLE:                    Isolation and identification of
                          1α-hydroxy-24-oxovitamin D3 and
                          1α,23-dihydroxy-24-oxovitamin D3 Metabolites of
                          1α,24(R)-dihydroxyvitamin D3 produced in rat
                          kidney
AUTHOR(S):                Weinstein, E. A.; Rao, D. S.; Siu-Caldera, M.-L.;
                          Tserng, K.-Y.; Uskokovic, M. R.; Ishizuka, S.; Reddy,
                          G. S.
CORPORATE SOURCE:         Women and Infants' Hospital of Rhode Island,
                          Department of Pediatrics, Brown University School of
                          Medicine, Providence, RI, USA
SOURCE:                   Biochemical Pharmacology (1999), 58(12),
                          1965-1973
                          CODEN: BCPCA6; ISSN: 0006-2952
PUBLISHER:                Elsevier Science Inc.
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
AB   1α,24(R)-Dihydroxyvitamin D3 [1α,24(R)(OH)2D3], a synthetic
     vitamin D3 analog, has been developed as a drug for topical use in the
     treatment of psoriasis.  At present, the target tissue metabolism of
     1α,24(R)(OH)2D3 is not understood completely.  In the authors'
     present study, the authors investigated the metabolism of
     1α,24(R)(OH)2D3 in the  isolated perfused rat kidney.  The
     results indicated that 1α,24(R)(OH)2D3 is metabolized in rat kidney
     into several metabolites, of which 1α,24(R),25-trihydroxyvitamin D3,
     1α,25-dihydroxy-24-oxovitamin D3, 1α,23(S),25-trihydroxy-24-
     oxovitamin D3, and 1α,23-dihydroxy-24,25,26,27-tetranorvitamin D3
     are similar to the previously known metabolites of 1α,25-
     dihydroxyvitamin D3 [1α,25(OH)2D3].  In addition to these
     aforementioned metabolites, the authors also identified two new
     metabolites, namely 1α-hydroxy-24-oxovitamin D3 and
     1α,23-dihydroxy-24-oxovitamin D3.  The two new metabolites do not
     possess the C-25 hydroxyl group.  Thus, the metabolism of
     1α,24(R)(OH)2D3 into both 25-hydroxylated and non-25-hydroxylated
     metabolites suggests that 1α,24(R)(OH)2D3 is metabolized in the rat
     kidney through two pathways.  The first pathway is initiated by C-25
     hydroxylation and proceeds further via the C-24 oxidation pathway.  The
     second pathway directly proceeds via the C-24 oxidation pathway without prior
     hydroxylation at the C-25 position.  Furthermore, the authors demonstrated
     that rat kidney did not convert 1α-hydroxyvitamin D3
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[1α(OH)D3] into 1α,25(OH)2D3. This finding indicates that the rat kidney does not possess the classical vitamin D3-25-hydroxylase (CYP27) activity. However, from the authors' present study it is apparent that prior hydroxylation of 1α(OH)D3 at the C-24 position in the 'R' orientation allows 25-hydroxylation to occur. At present, the enzyme responsible for the C-25 hydroxylation of 1α,24(R)(OH)2D3 is unknown. The authors' observation that the side chain of 1α,24(R)(OH)2D3 underwent 24-ketonization and 23-hydroxylation even in the absence of the C-25 hydroxyl group suggests that 1α,25(OH)2D3-24-hydroxylase (CYP24) can perform some steps of the C-24 oxidation pathway without prior C-25 hydroxylation. Thus, the authors speculate that CYP24 may be playing a dual role in the metabolism of 1α,24(R)(OH)2D3.

REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 2 OF 8 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1998:485824 HCAPLUS
DOCUMENT NUMBER: 129:198413
TITLE: Induction of the vitamin D 24-hydroxylase (CYP24) by 1,25-dihydroxyvitamin D3 is regulated by parathyroid hormone in UMR106 osteoblastic cells
AUTHOR(S): Armbrecht, H. J.; Hodam, T. L.; Boltz, M. A.; Partridge, N. C.; Brown, A. J.; Kumar, V. B.
CORPORATE SOURCE: Geriatric Research, Education & Clinical Center, St. Louis VA Med. Center, St. Louis, MO, 63125, USA
SOURCE: Endocrinology (1998), 139(8), 3375-3381
CODEN: ENDOAO; ISSN: 0013-7227
PUBLISHER: Endocrine Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The expression of the vitamin D 24-hydroxylase is highly regulated in target tissues for 1,25-dihydroxyvitamin D3 (1,25(OH)2D), where it may modulate the action of 1,25(OH)2D. In UMR106 osteoblastic cells, 1,25(OH)2D and PTH synergistically induce 24-hydroxylase expression. The purpose of these studies was to characterize the interaction between 1,25(OH)2D and PTH with regard to the mRNA levels of the cytochrome P 450 component of the 24-hydroxylase (CYP24). PTH alone had no effect on CYP24 mRNA levels, and 1,25(OH)2D alone produced only a modest increase. However, 1,25(OH)2D and PTH together synergistically increased CYP24 mRNA levels 3-fold compared with 1,25(OH)2D alone. PTH also increased the sensitivity of UMR cells to 1,25(OH)2D from 10-8 to 10-10 M. PTH worked through the cAMP signaling pathway as evidenced by the lack of effect of PTH (3-34) and by the full activity of 8-bromo-cAMP. PTH in the presence of 1,25(OH)2D increased CYP24 gene transcription as shown by nuclear run-on studies and by activation of a CYP24 promoter-reporter construct after transfection. PTH also increased vitamin D receptor number in UMR cells, but this occurred at times later than the increase in transcription. These studies demonstrate that PTH in the presence of 1,25(OH)2D works through the cAMP-dependent signaling pathway to increase transcription of the CYP24 gene, to increase CYP24 protein levels, and to increase 24-hydroxylase activity.

REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 3 OF 8 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1997:712383 HCAPLUS

DOCUMENT NUMBER: 128:10692
TITLE: Molecular cloning of vitamin D3 hydroxylases
AUTHOR(S): Noshiro, Mitsuhide; Ohyama, Yoshihiko; Usui, Emiko; Akiyoshi-Shibata, Megumi; Yabusaki, Yoshiyasu; Okuda, Kyu-Ichiro
CORPORATE SOURCE: USA
SOURCE: Methods in Enzymology (1997), 282(Vitamins and Coenzymes, Part L), 213-223
CODEN: MENZAU; ISSN: 0076-6879
PUBLISHER: Academic
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review, with 34 refs., on the mol. cloning of cDNAs for vitamin D3 25-hydroxylase (CYP27) and 25-hydroxyvitamin D3 24-hydroxylase (CYP24) from rat in COS-7, yeast, and Escherichia coli cells.
REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 4 OF 8 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1997:680570 HCAPLUS
DOCUMENT NUMBER: 127:355817
TITLE: The 25-hydroxyvitamin D 1-alpha-hydroxylase gene maps to the pseudovitamin D-deficiency rickets (PDDR) disease locus
AUTHOR(S): St-Arnaud, Rene; Messerlian, Serge; Moir, Janet M.; Omdahl, John L.; Glorieux, Francis H.
CORPORATE SOURCE: Genetics Unit, Shriners Hospital, McGill University, Montreal, QC, Can.
SOURCE: Journal of Bone and Mineral Research (1997), 12(10), 1552-1559
CODEN: JBMREJ; ISSN: 0884-0431
PUBLISHER: Blackwell
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Pseudovitamin D-deficiency rickets (PDDR) is an autosomal recessive disorder that may be due to impaired activity of 25-hydroxyvitamin D-1α-hydroxylase, a renal cytochrome P 450 enzyme (P 4501α) of the vitamin D pathway. The disease locus for PDDR has been mapped by linkage anal. to 12q13-q14, but the mol. defect underlying the enzyme dysfunction has remained elusive due to the lack of sequence information for the P 4501α, gene (hereafter referred to as 1α-OHase). A probe derived from the rat 25-hydroxyvitamin D-24-hydroxylase (CYP24; 24-OHase) sequence was used to identify and clone the 1α-OHase cDNA. The full-length 1α-OHase clone of 2.4 kb codes for a protein of predicted Mr 55 kDa. Functional activity of the cloned sequence was assessed using transient transfection, and the production of authentic 1α,25-dihydroxyvitamin D3 [1α,25(OH)2D3] was confirmed using high performance liquid chromatog. fractionation and time-of-flight mass spectrometry. The expression of the gene was analyzed in vitamin D-replete animals; treatment with 1α,25(OH)2D3 reduced 1α-OHase transcript levels by 70%, while administration of parathyroid hormone led to a 2-fold increase in the expression of the gene, thus confirming the hormonal regulation previously described using biochem. methods. The rat cDNA was used to obtain a human genomic clone. Interestingly, the human 1α-OHase gene mapped to 12q13.1-q13.3, providing strong evidence that a mutation in the 1α-OHase gene is responsible for the PDDR phenotype. The availability of a cloned sequence for 1α-OHase generates novel tools for the study of the mol. etiol. of PDDR, and will allow the investigation of other disturbances of vitamin

D metabolism
REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 5 OF 8 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1995:865271 HCAPLUS
DOCUMENT NUMBER: 124:24692
TITLE: Molecular cloning of 25-hydroxyvitamin D-3 24-hydroxylase (Cyp-24) from mouse kidney: its inducibility by vitamin D-3
AUTHOR(S): Itoh, Susumu; Yoshimura, Takuya; Iemura, Osamu; Yamada, Eitaro; Tsujikawa, Kazutake; Kohama, Yasuhiro; Mimura, Tsutomu
CORPORATE SOURCE: Division of Bio-Medical and Immunological Chemistry, Faculty of Pharmaceutical Sciences, Osaka University, 1-6, Yamadaoka, Suita, Osaka, 565, Japan
SOURCE: Biochimica et Biophysica Acta, Gene Structure and Expression (1995), 1264(1), 26-8
CODEN: BBGSD5; ISSN: 0167-4781
PUBLISHER: Elsevier B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A cDNA encoding a 25-hydroxyvitamin D-3 24-hydroxylase, Cyp-24, has been isolated from mouse kidney cDNA library by hybridization screening. Mouse Cyp-24, coding for 514 amino acid residues, shared 82.1 and 94.7% amino acid identity with human and rat CYP24s, resp. Among mouse organs examined, Cyp-24 mRNA could be detected in the kidney. When mice were treated with vitamin D-3, Cyp-24 mRNA was induced in the kidney.

L10 ANSWER 6 OF 8 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1995:697760 HCAPLUS
DOCUMENT NUMBER: 123:163984
TITLE: Different mechanisms of hydroxylation site selection by liver and kidney cytochrome P450 species (CYP27 and CYP24) involved in vitamin D metabolism
AUTHOR(S): Dilworth, F. Jeffrey; Scott, Ian; Green, Andrew; Strugnell, Stephen; Guo, Yu-Ding; Roberts, Eve A.; Kremer, Richard; Calverley, Martin J.; Makin, Hugh L. J.; Jones, Glenville
CORPORATE SOURCE: Dep. Biochem., Queen's Univ., Kingston, ON, K7L 3N6, Can.
SOURCE: Journal of Biological Chemistry (1995), 270(28), 16766-74
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Bio logy
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A series of homologated 1α-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 mols. with one to three extra carbons in the side chain were used to examine the substrate preferences and hydroxylation site selection mechanisms of the liver vitamin D3-25-hydroxylase (CYP27) and the target cell 25-hydroxyvitamin D3-24-hydroxylase (CYP24). Cultured and transfected cell models, used as sources of these hydroxylases, gave 23-, 24-, 25-, and 27-hydroxylated metabolites which were identified by their high performance liquid chromatog. and GC-MS characteristics. Lengthening the side chain is tolerated by each cytochrome P 450 isoform such that 25-hydroxylation or 24-hydroxylation continues to occur at the same rate as in the native side chain, while the

site of hydroxylation remains the same for the liver enzyme in that CYP27 continues to hydroxylate at C-25 and C-27 (minor) despite the two-carbon-atom extension. Somewhat surprising is the finding that C-24 and C23 (minor) hydroxylations also do not change as the side chain is extended by as much as three carbons. The authors conclude that CYP24 must be directed to its hydroxylation site(s) by the distance of carbon 24 from the vitamin D ring structure and not as in CYP27 by the distance of the hydroxylation site from the end of the side chain.

L10 ANSWER 7 OF 8 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1995:138056 HCAPLUS
DOCUMENT NUMBER: 122:152520
TITLE: The genes for endothelin 3, vitamin D 24-hydroxylase, and melanocortin 3 receptor map to distal mouse chromosome 2, in the region of conserved synteny with human chromosome 20
AUTHOR(S): Malas, S.; Peters, J.; Abbott, C.
CORPORATE SOURCE: Department of Genetics and Biometry, University College London, London, NW1 2HE, UK
SOURCE: Mammalian Genome (1994), 5(9), 577-9
CODEN: MAMGEC; ISSN: 0938-8990
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Described is the mapping of the mouse homologs of 3 genes that map to the distal long arm of human chromosome 20, the genes for vitamin D 24-hydroxylase (CYP24), endothelin 3 (EDN3), and melanocortin 3 receptor (MC3R).

L10 ANSWER 8 OF 8 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1993:421987 HCAPLUS
DOCUMENT NUMBER: 119:21987
TITLE: Localization of the human vitamin D 24-hydroxylase gene (CYP24) to chromosome 20q13.2→q13.3
AUTHOR(S): Hahn, C. N.; Baker, E.; Laslo, P.; May, B. K.; Omdahl, J. L.; Sutherland, G. R.
CORPORATE SOURCE: Dep. Biochem., Univ. Adelaide, Adelaide, Australia
SOURCE: Cytogenetics and Cell Genetics (1993), 62(4), 192-3
CODEN: CGCGBR; ISSN: 0301-0171
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The gene encoding human vitamin D 24-hydroxylase (P 45024) has been localized by fluorescence in situ hybridization to 20q13.2→q13.3.

```
=> d que stat l14
L1             2 SEA FILE=REGISTRY ABB=ON  ("VITAMIN D 24-HYDROXYLASE (MOUSE
                  PRECURSOR)"/CN OR "VITAMIN D 25-HYDROXYLASE"/CN)
L2           302 SEA FILE=HCAPLUS ABB=ON  L1 OR ?VITAMIN?(W)D(W)24(W)?HYDROXYLAS
                  E?
L3            26 SEA FILE=HCAPLUS ABB=ON  L2 AND CYP24
L4            10 SEA FILE=HCAPLUS ABB=ON  L3 AND MRNA
L5            26 SEA FILE=HCAPLUS ABB=ON  L3 OR L4
L6             8 SEA FILE=HCAPLUS ABB=ON  L5 AND ?PROTEIN?
L7            26 SEA FILE=HCAPLUS ABB=ON  L5 OR L6
L8             9 SEA FILE=HCAPLUS ABB=ON  L7 AND (?DETECT? OR ?IDENT? OR
                  ?ISOLAT?)
L9            26 SEA FILE=HCAPLUS ABB=ON  L7 OR L8
L11          119 SEA L9
L12           92 DUP REMOV L11 (27 DUPLICATES REMOVED)
L13           41 SEA L12 AND (MRNA OR RECOMB?)
L14           21 SEA L13 AND ?PROTEIN?

=> d ibib abs l14 1-21

L14  ANSWER 1 OF 21     MEDLINE on STN
ACCESSION NUMBER:   2006143127     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 16483768
TITLE:              Inhibition of Vitamin D3 metabolism enhances VDR signalling
                    in androgen-independent prostate cancer cells.
AUTHOR:             Yee Sook Wah; Campbell Moray J; Simons Claire
CORPORATE SOURCE:   Division of Medicinal Chemistry, Welsh School of Pharmacy,
                    Cardiff University, UK.
SOURCE:             The Journal of steroid biochemistry and molecular biology,
                    (2006 Mar) Vol. 98, No. 4-5, pp. 228-35.  Electronic
                    Publication: 2006-02-14.
                    Journal code: 9015483. ISSN: 0960-0760.
PUB. COUNTRY:       England: United Kingdom
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200604
ENTRY DATE:         Entered STN: 14 Mar 2006
                    Last Updated on STN: 26 Apr 2006
                    Entered Medline: 25 Apr 2006
AB   Induction of growth arrest and differentiation by 1alpha,25-
     dihydroxyvitamin D(3) (1,25-(OH)(2)D(3)) occurs in non-malignant cell
     types but is often reduced in cancer cells.  For example,
     androgen-independent prostate cancer cells, DU-145 and PC-3, are
     relatively insensitive to the anti-proliferative action of
     1,25-(OH)(2)D(3).  This appears to be due to increased
     1,25-(OH)(2)D(3)-metabolism, as a result of CYP24
     enzyme-induction, which in turn leads to decreased anti-proliferative
     efficacy.  In the in vitro rat kidney mitochondria assay, the
     2-(4-hydroxybenzyl)-6-methoxy-3,4-dihydro-2H-naphthalen-1-one (4) was
     found to be a potent inhibitor of Vitamin D(3) metabolising enzymes
     (IC(50) 3.5 microM), and was shown to be a more potent inhibitor than the
     broad spectrum P450 inhibitor ketoconazole (IC(50) 20 microM).  The
     combination of the inhibitor and 1,25-(OH)(2)D(3) caused a greater
     inhibition of proliferation in DU-145 cells than when treated with both
     agents alone.  Examination of the regulation of VDR target gene
     mRNA in DU-145 cells revealed that co-treatment of
     1,25-(OH)(2)D(3) plus inhibitor of Vitamin D(3) metabolising enzymes
     co-ordinately upregulated CYP24, p21(waf1/cip1) and GADD45alpha.
```

L14 ANSWER 2 OF 21 MEDLINE on STN
ACCESSION NUMBER: 2005484446 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15955619
TITLE: Genistein potentiates the growth inhibitory effects of 1,25-dihydroxyvitamin D3 in DU145 human prostate cancer cells: role of the direct inhibition of CYP24 enzyme activity.
AUTHOR: Swami Srilatha; Krishnan Aruna V; Peehl Donna M; Feldman David
CORPORATE SOURCE: Department of Medicine, Division of Endocrinology, Stanford University School of Medicine, 300 Pasteur Dr, Stanford, CA 94305, USA.
CONTRACT NUMBER: AT00486 (NCCAM)
CA92238 (NCI)
DK42482 (NIDDK)
SOURCE: Molecular and cellular endocrinology, (2005 Sep 28) Vol. 241, No. 1-2, pp. 49-61.
Journal code: 7500844. ISSN: 0303-7207.
PUB. COUNTRY: Ireland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200512
ENTRY DATE: Entered STN: 13 Sep 2005
Last Updated on STN: 31 Dec 2005
Entered Medline: 30 Dec 2005

AB In a search for improved therapies for prostate cancer, we investigated the effect of genistein in combination with 1alpha-25-dihydroxyvitamin D3 [1,25(OH)2D3], on the growth of DU145 human prostate cancer cells. DU145 cells were very resistant to the growth inhibitory action of 1,25(OH)2D3 or genistein when administered individually. However, the combination caused a significant growth inhibition seen at lower concentrations of both agents. 1,25(OH)2D3 induces the expression of the CYP24 gene, which codes for the enzyme that initiates the catabolism of 1,25(OH)2D3. We showed for the first time that genistein at low doses (50-100 nM) directly inhibited CYP24 at the enzyme level. Addition of genistein to mitochondrial preparations inhibited CYP24 enzyme activity in a noncompetitive manner. CYP24 inhibition by genistein increased the half-life of 1,25(OH)2D3 thereby augmenting the homologous up-regulation of the vitamin D receptor (VDR) both at the mRNA and protein levels. Genistein co-treatment enhanced 1,25(OH)2D3-mediated transactivation of the vitamin D responsive reporters OC-Luc and OP-Luc transfected into DU145 cells. Consistent with the growth inhibition due to the combination treatment, significant changes in the expression of genes involved in growth arrest and apoptosis were seen. We conclude that genistein potentiates the antiproliferative actions of 1,25(OH)2D3 in DU145 cells by two mechanisms: (i) an increase in the half-life of 1,25(OH)2D3 due to the direct inhibition of CYP24 enzyme activity and (ii) an amplification of the homologous up-regulation of VDR. Together these two effects lead to a substantial enhancement of the cellular responses to the growth inhibitory and pro-apoptotic signaling by 1,25(OH)2D3.

L14 ANSWER 3 OF 21 MEDLINE on STN
ACCESSION NUMBER: 2005400691 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16061850
TITLE: Vitamin D(3) metabolism in human glioblastoma multiforme: functionality of CYP27B1 splice variants, metabolism of calcidiol, and effect of calcitriol.
AUTHOR: Diesel Britta; Radermacher Jens; Bureik Matthias; Bernhardt

Rita; Seifert Markus; Reichrath Jorg; Fischer Ulrike; Meese Eckart
CORPORATE SOURCE: Institut fur Humangenetik, Theoretische Medizin, Universitat des Saarlandes, Saarbrucken, Germany.
SOURCE: Clinical cancer research : an official journal of the American Association for Cancer Research, (2005 Aug 1) Vol. 11, No. 15, pp. 5370-80.
Journal code: 9502500. ISSN: 1078-0432.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200601
ENTRY DATE: Entered STN: 3 Aug 2005
Last Updated on STN: 6 Jan 2006
Entered Medline: 5 Jan 2006

AB PURPOSE: A better understanding of the vitamin D(3) metabolism is required to evaluate its potential therapeutic value for cancers. Here, we set out to contribute to the understanding of vitamin D(3) metabolism in glioblastoma multiforme. EXPERIMENTAL DESIGN: We did nested touchdown reverse transcription-PCR (RT-PCR) to identify CYP27B1 splice variants and real-time RT-PCR to quantify the expression of CYP27B1. A cell line was treated with calcitriol to determine the effect on the expression of CYP27B1, 1alpha,25-dihydroxyvitamin D(3)-24-hydroxylase (CYP24), and vitamin D(3) receptor (VDR). We generated three antibodies for the specific detection of CYP27B1 and splice variants. High-performance TLC was done to determine the endogenous CYP27B1 activity and the functionality of CYP27B1 splice variants. Using WST-1 assay, we determined the effect of vitamin D(3) metabolites on proliferation. RESULTS: We report a total of 16 splice variants of CYP27B1 in glioblastoma multiforme and a different expression of CYP27B1 and variants between glioblastoma multiforme and normal tissues. We found preliminary evidence for enzymatic activity of endogenous CYP27B1 in glioblastoma multiforme cell cultures but not for the functionality of the splice variants. By adding calcitriol, we found a proliferative effect for some cell lines depending on the dose of calcitriol. The administration of calcitriol led to an elevated expression of CYP27B1 and CYP24 but left the expression of the VDR unaltered. CONCLUSIONS: Our findings show that glioblastoma multiforme cell lines metabolize calcidiol. In addition, we show various effects mediated by calcitriol. We found a special vitamin D(3) metabolism and mode of action in glioblastoma multiforme that has to be taken into account in future vitamin D(3)-related therapies.

L14 ANSWER 4 OF 21 MEDLINE on STN
ACCESSION NUMBER: 2005383211 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15860548
TITLE: Gene regulatory potential of nonsteroidal vitamin D receptor ligands.
AUTHOR: Perakyla Mikael; Malinen Marjo; Herzig Karl-Heinz; Carlberg Carsten
CORPORATE SOURCE: Department of Chemistry, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland.
SOURCE: Molecular endocrinology (Baltimore, Md.), (2005 Aug) Vol. 19, No. 8, pp. 2060-73. Electronic Publication: 2005-04-28.
Journal code: 8801431. ISSN: 0888-8809.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 200512
ENTRY DATE: Entered STN: 26 Jul 2005
Last Updated on STN: 15 Dec 2005
Entered Medline: 2 Dec 2005

AB The seco-steroid 1alpha,25-dihydroxyvitamin D3 [1alpha,25(OH)2D3] is a promising drug candidate due to its pleiotropic function including the regulation of calcium homeostasis, bone mineralization and cellular proliferation, differentiation, and apoptosis. We report here a novel class of nonsteroidal compounds, represented by the bis-aromatic molecules CD4409, CD4420, and CD4528, as ligands of the 1alpha,25(OH)2D3 receptor (VDR). Taking the known diphenylmethane derivative LG190178 as a reference, this study provides molecular evaluation of the interaction of nonsteroidal ligands with the VDR. All four nonsteroidal compounds were shown to induce VDR-retinoid X receptor heterodimer complex formation on a 1alpha,25(OH)2D3 response element, stabilize the agonistic conformation of the VDR ligand-binding domain, enable the interaction of VDR with coactivator proteins and contact with their three hydroxyl groups the same residues within the ligand-binding pocket of the VDR as 1alpha,25(OH)2D3. Molecular dynamics simulations demonstrated that all four nonsteroidal ligands take a shape within the ligand-binding pocket of the VDR that is very similar to that of the natural ligand. CD4528 is mimicking the natural hormone best and was found to be in vitro at least five times more potent than LG190178. In living cells, CD4528 was only two times less potent than 1alpha,25(OH)2D3 and induced mRNA expression of the VDR target gene CYP24 in a comparable fashion. At a noncalcemic dose of 150 microg/kg, CD4528 showed in vivo a clear induction of CYP24 expression and therefore may be used as a lead compound for the development of therapeutics against psoriasis, osteoporosis, and cancer.

L14 ANSWER 5 OF 21 MEDLINE on STN
ACCESSION NUMBER: 2005251739 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15890193
TITLE: The human peroxisome proliferator-activated receptor delta gene is a primary target of 1alpha,25-dihydroxyvitamin D3 and its nuclear receptor.
AUTHOR: Dunlop Thomas W; Vaisanen Sami; Frank Christian; Molnar Ferdinand; Sinkkonen Lasse; Carlberg Carsten
CORPORATE SOURCE: Department of Biochemistry, University of Kuopio, FIN-70211 Kuopio, Finland.
SOURCE: Journal of molecular biology, (2005 Jun 3) Vol. 349, No. 2, pp. 248-60. Electronic Publication: 2005-04-07.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200506
ENTRY DATE: Entered STN: 14 May 2005
Last Updated on STN: 22 Jun 2005
Entered Medline: 21 Jun 2005

AB Peroxisome proliferator-activated receptor (PPAR) delta is the most widely expressed member of the PPAR family of nuclear receptor fatty acid sensors. Real-time PCR analysis of breast and prostate cancer cell lines demonstrated that PPARdelta expression was increased 1.5 to 3.2-fold after three hours stimulation with the natural vitamin D receptor (VDR) agonist, 1alpha,25-dihydroxyvitamin D3 (1alpha,25(OH)2D3). In silico analysis of the 20 kb of the human PPARdelta promoter revealed a DR3-type 1alpha,25(OH)2D3 response element approximately 350 bp upstream of the

transcription start site, which was able to bind VDR-retinoid X receptor (RXR) heterodimers and mediate a 1alpha,25(OH)2D3-dependent upregulation of reporter gene activity. Chromatin immuno-precipitation assays demonstrated that a number of proteins representative for 1alpha,25(OH)2D3-mediated gene activation, such as VDR, RXR and RNA polymerase II, displayed a 1alpha,25(OH)2D3-dependent association with a region of the proximal PPARdelta promoter that contained the putative DR3-type VDRE. This was also true for other proteins that are involved in or are the subject of chromatin modification, such as the histone acetyltransferase CBP and histone 4, which displayed ligand-dependent association and acetylation, respectively. Finally, real-time PCR analysis demonstrated that 1alpha,25(OH)2D3 and the synthetic PPARdelta ligand L783483 show a cell and time-dependent interference in each other's effects on VDR mRNA expression, so that their combined application shows complex effects on the induction of VDR target genes, such as CYP24. Taken together, we conclude that PPARdelta is a primary 1alpha,25(OH)2D3-responding gene and that VDR and PPARdelta signaling pathways are interconnected at the level of cross-regulation of their respective transcription factor mRNA levels.

L14 ANSWER 6 OF 21 MEDLINE on STN
ACCESSION NUMBER: 2005229535 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15863722
TITLE: Regulation of the human cyclin C gene via multiple vitamin D3-responsive regions in its promoter.
AUTHOR: Sinkkonen Lasse; Malinen Marjo; Saavalainen Katri; Vaisanen Sami; Carlberg Carsten
CORPORATE SOURCE: Department of Biochemistry, University of Kuopio PO Box 1627, FIN-70211 Kuopio, Finland.
SOURCE: Nucleic acids research, (2005) Vol. 33, No. 8, pp. 2440-51. Electronic Publication: 2005-04-29. Journal code: 0411011. E-ISSN: 1362-4962.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200505
ENTRY DATE: Entered STN: 3 May 2005
Last Updated on STN: 13 May 2005
Entered Medline: 12 May 2005

AB The candidate human tumor suppressor gene cyclin C is a primary target of the anti-proliferative hormone 1alpha,25-dihydroxyvitamin D3 [1alpha,25(OH)2D3], but binding sites for the 1alpha,25(OH)2D3 receptor (VDR), so-called 1alpha,25(OH)2D3 response elements (VDREs), have not yet been identified in the promoter of this gene. We screened various cancer cell lines by quantitative PCR and found that the 1alpha,25(OH)2D3 inducibility of cyclin C mRNA expression, in relationship with the 24-hydroxylase (CYP24) gene, was best in MCF-7 human breast cancer cells. To characterize the molecular mechanisms, we analyzed 8.4 kb of the cyclin C promoter by using chromatin immunoprecipitation assays (ChIP) with antibodies against acetylated histone 4, VDR and its partner receptor, retinoid X receptor (RXR). The histone 4 acetylation status of all 23 investigated regions of the cyclin C promoter did not change significantly in response to 1alpha,25(OH)2D3, but four independent promoter regions showed a consistent, 1alpha,25(OH)2D3-dependent association with VDR and RXR over a time period of 240 min. Combined in silico/in vitro screening identified in each of these promoter regions a VDRE and reporter gene assays confirmed their functionality. Moreover, re-ChIP assays monitored simultaneous

association of VDR with RXR, coactivator, mediator and RNA polymerase II proteins on these regions. Since cyclin C protein is associated with those mediator complexes that display transcriptional repressive properties, this study contributes to the understanding of the downregulation of a number of secondary 1alpha,25(OH)2D3-responding genes.

L14 ANSWER 7 OF 21 MEDLINE on STN
ACCESSION NUMBER: 2004324609 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15225765
TITLE: Affinity labeling of rat cytochrome P450C24 (CYP24) and identification of Ser57 as an active site residue.
AUTHOR: Omdahl J L; Swamy N; Serda R; Annalora A; Berne M; Rayb R
CORPORATE SOURCE: University of New Mexico School of Medicine, Albuquerque, NM 87131, USA.
SOURCE: The Journal of steroid biochemistry and molecular biology, (2004 May) Vol. 89-90, No. 1-5, pp. 159-62. Journal code: 9015483. ISSN: 0960-0760.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200408
ENTRY DATE: Entered STN: 1 Jul 2004
Last Updated on STN: 1 Sep 2004
Entered Medline: 31 Aug 2004

AB 25-hydroxyvitamin D(3)- or 1alpha,25-dihydroxyvitamin D(3)-24R-hydroxylase (cytochromeP450C24 or CYP24) has a dual role of removing 25-OH-D(3) from circulation and excess 1,25(OH)(2)D(3) from kidney. As a result, CYP24 is an important multifunctional regulatory enzyme that maintains essential tissue-levels of Vitamin D hormone. As a part of our continuing interest in structure-function studies characterizing various binding proteins in the Vitamin D endocrine system, we targeted recombinant rat CYP24 with a radiolabeled 25-OH-D(3) affinity analog, and showed that the 25-OH-D(3)-binding site was specifically labeled by this analog. An affinity labeled sample of CYP24 was subjected to MS/MS analysis, which identified Ser57 as the only amino acid residue in the entire length of the protein that was covalently modified by this analog. Site-directed mutagenesis was conducted to validate the role of Ser57 towards substrate-binding. S57A mutant displayed significantly lower binding capacity for 25-OH-D(3) and 1,25(OH)(2)D(3). On the other hand, S57D mutant strongly enhanced binding for the substrates and conversion of 1,25(OH)(2)D(3) to calcitroic acid. The affinity probe was anchored via the 3-hydroxyl group of 25-OH-D(3). Therefore, these results suggested that the 3-hydroxyl group (of 25-OH-D(3) and 1,25(OH)(2)D(3)) in the S57D mutant could be stabilized by hydrogen bonding or a salt bridge leading to enhanced substrate affinity and metabolism.

L14 ANSWER 8 OF 21 MEDLINE on STN
ACCESSION NUMBER: 2003438018 MEDLINE
DOCUMENT NUMBER: PubMed ID: 13679056
TITLE: Metabolism of 20-epimer of 1alpha,25-dihydroxyvitamin D3 by CYP24: species-based difference between humans and rats.
AUTHOR: Kusudo Tatsuya; Sakaki Toshiyuki; Abe Daisuke; Fujishima Toshie; Kittaka Atsushi; Takayama Hiroaki; Ohta Miho; Inouye Kuniyo
CORPORATE SOURCE: Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University Kitashirakawa, Oiwake-cho,

Sakyo-ku, Kyoto 606-8502, Japan.
SOURCE: Biochemical and biophysical research communications, (2003 Oct 3) Vol. 309, No. 4, pp. 885-92.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200312
ENTRY DATE: Entered STN: 23 Sep 2003
Last Updated on STN: 18 Dec 2003
Entered Medline: 4 Dec 2003

AB The 20-epi form of 1alpha,25-dihydroxyvitamin D(3) (1alpha,25(OH)(2)-20-epi-D(3)) is expected as drugs for leukemia, other cancers or psoriasis, because it shows several-hundred fold enhanced ability to induce cell differentiation and growth inhibition than 1alpha,25-dihydroxyvitamin D(3) while its calcemic activity is only slightly elevated. In this study, we compared the human and rat CYP24-dependent metabolism of 1alpha,25(OH)(2)-20-epi-D(3) by using the Escherichia coli expression system. The HPLC and LC-MS analyses of the metabolites revealed that rat CYP24 converted 1alpha,25(OH)(2)-20-epi-D(3) to 25,26,27-trinor-1alpha(OH)-24(COOH)-20-epi-D(3) through 1alpha,24,25(OH)(3)-20-epi-D(3) and 1alpha,25(OH)(2)-24-oxo-20-epi-D(3). The binding affinity of trinor-1alpha(OH)-24(COOH)-20-epi-D(3) for vitamin D receptor (VDR) was less than 1/4000 of that of 1alpha,25(OH)(2)-20-epi-D(3). These results suggest that rat CYP24 can almost completely inactivate 1alpha,25(OH)(2)-20-epi-D(3). On the other hand, human CYP24 mainly converted 1alpha,25(OH)(2)-20-epi-D(3) to its putative demethylated compound with a hydroxyl group, via 1alpha,24,25(OH)(3)-20-epi-D(3), 1alpha,25(OH)(2)-24-oxo-20-epi-D(3), and 1alpha,23,25(OH)(3)-24-oxo-20-epi-D(3). All of these metabolites showed considerable affinity for vitamin D receptor. These results clearly demonstrate the species-based difference between human and rat on the CYP24-dependent metabolism of 1alpha,25(OH)(2)-20-epi-D(3).

L14 ANSWER 9 OF 21 MEDLINE on STN
ACCESSION NUMBER: 2003371010 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12846736
TITLE: Gene expression of vitamin D hydroxylase and megalin in the remnant kidney of nephrectomized rats.
AUTHOR: Takemoto Fumi; Shinki Toshimasa; Yokoyama Keitaro; Inokami Taketoshi; Hara Shigeko; Yamada Akira; Kurokawa Kiyoshi; Uchida Shunya
CORPORATE SOURCE: Kidney Center, Toranomon Hospital, Tokyo, Japan.. ftakemoto-ind@umin.ac.jp
SOURCE: Kidney international, (2003 Aug) Vol. 64, No. 2, pp. 414-20.
Journal code: 0323470. ISSN: 0085-2538.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200403
ENTRY DATE: Entered STN: 9 Aug 2003
Last Updated on STN: 27 Mar 2004
Entered Medline: 26 Mar 2004

AB BACKGROUND: Regulation of vitamin D hydroxylase genes in the early stage of chronic renal failure is not fully understood. Using nephrectomized rats, we examined changes in mRNA levels of CYP27B1 (25-hydroxyvitamin D3-1 alpha-hydroxylase), CYP24

(25-hydroxyvitamin D3-24-hydroxylase), and vitamin D receptor in relation to megalin, recently found to participate in renal vitamin D metabolism. METHODS: A rat model of moderate renal failure was induced by 3/4 nephrectomy. Plasma parameters, including vitamin D metabolite concentrations, were measured at weeks 2, 4 and 8, and poly(A)+ RNA extracted from the remnant kidneys was subjected to Northern blot hybridization. RESULTS: Plasma creatinine concentration at week 2 was 0.40 +/- 0.02 mg/dL in the sham-operated and 0.93 +/- 0.15 mg/dL in the nephrectomized rats, and both values remained constant up to week 8. Plasma concentrations of 25(OH)D3, 1 alpha,25(OH)2D3, and 24,25(OH)2D3 were unchanged between nephrectomized and sham-operated rats at week 8. Intact parathyroid hormone (PTH) increased at week 8 in nephrectomized rats. CYP27B1 mRNA in nephrectomized rats did not vary at week 2, but increased approximately two- and four-fold at weeks 4 and 8, respectively, compared to the sham-operated rats. CYP24 and megalin mRNAs, on the other hand, began to decline as early as at week 2 in nephrectomized rats and kept decreasing throughout the experiment. The expression of vitamin D receptor was modestly but significantly decreased only at week 8. CONCLUSION: Coordinated and reciprocal alterations of the increase in CYP27B1 mRNA and the decrease in CYP24 mRNA may play a pivotal role in maintaining the plasma level of 1 alpha,25(OH)2D3 in the face of reduced nephron mass and/or megalin expression.

L14 ANSWER 10 OF 21 MEDLINE on STN
ACCESSION NUMBER: 2003261004 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12787875
TITLE: Metabolism of 26,26,26,27,27,27-F6-1alpha,25-dihydroxyvitamin D3 by CYP24: species-based difference between humans and rats.
AUTHOR: Sakaki Toshiyuki; Sawada Natsumi; Abe Daisuke; Komai Koichiro; Shiozawa Shunichi; Nonaka Yasuki; Nakagawa Kimie; Okano Toshio; Ohta Miho; Inouye Kuniyo
CORPORATE SOURCE: Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kitashirakawa, Oiwake-cho, Sakyo-ku, Japan.. tsakaki@kais.kyoto-u.ac.jp
SOURCE: Biochemical pharmacology, (2003 Jun 15) Vol. 65, No. 12, pp. 1957-65.
Journal code: 0101032. ISSN: 0006-2952.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200307
ENTRY DATE: Entered STN: 6 Jun 2003
Last Updated on STN: 23 Jul 2003
Entered Medline: 22 Jul 2003

AB The compound 26,26,26,27,27,27-F(6)-1alpha,25(OH)(2)D(3) is a hexafluorinated analog of the active form of Vitamin D(3). The enhanced biological activity of F(6)-1alpha,25(OH)(2)D(3) is considered to be related to a decreased metabolic inactivation of the compound in target tissues such as the kidneys, small intestine, and bones. Our previous study demonstrated that CYP24 is responsible for the metabolism of F(6)-1alpha,25(OH)(2)D(3) in the target tissues. In this study, we compared the human and rat CYP24-dependent metabolism of F(6)-1alpha,25(OH)(2)D(3) by using the Escherichia coli expression system. In the recombinant E. coli cells expressing human CYP24 , bovine adrenodoxin and NADPH-adrenodoxin reductase, F(6)-1alpha,25(OH)(2)D(3) was successively converted to F(6)-1alpha,23S,25(OH)(3)D(3), F(6)-23-oxo-1alpha,25(OH)(2)D(3), and the

putative ether compound with the same molecular mass as F(6)-1alpha,25(OH)(2)D(3). The putative ether was not observed in the recombinant E. coli cells expressing rat CYP24. These results indicate species-based difference between human and rat CYP24 in the metabolism of F(6)-1alpha,25(OH)(2)D(3). In addition, the metabolite with a cleavage at the C(24)z.sbnd;C(25) bond of F(6)-1alpha,25(OH)(2)D(3) was detected as a minor metabolite in both human and rat CYP24. Although F(6)-1alpha,23S,25(OH)(3)D(3) and F(6)-23-oxo-1alpha,25(OH)(2)D(3) had a high affinity for Vitamin D receptor, the side-chain cleaved metabolite and the putative ether showed extremely low affinity for Vitamin D receptor. These findings indicate that human CYP24 has a dual pathway for metabolic inactivation of F(6)-1alpha,25(OH)(2)D(3) while rat CYP24 has only one pathway. Judging from the fact that metabolism of F(6)-1alpha,25(OH)(2)D(3) in rat CYP24-harboring E. coli cells is quite similar to that in the target tissues of rat, the metabolism seen in human CYP24-harboring E. coli cells appear to exhibit the same metabolism as in human target tissues. Thus, this recombinant system harboring human CYP24 appears quite useful for predicting the metabolism and efficacy of Vitamin D analogs in human target tissues before clinical trials.

L14 ANSWER 11 OF 21 MEDLINE on STN
ACCESSION NUMBER: 2002741104 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12504896
TITLE: Hormonal regulation of 25-hydroxyvitamin D3-1alpha-hydroxylase and 24-hydroxylase gene transcription in opossum kidney cells.
AUTHOR: Armbrecht H J; Hodam T L; Boltz M A
CORPORATE SOURCE: Geriatric Research, Education, and Clinical Center (11G-JB), St. Louis VA Medical Center, St. Louis, MO 63125, USA.. hjarmbrec@aol.com
SOURCE: Archives of biochemistry and biophysics, (2003 Jan 15) Vol. 409, No. 2, pp. 298-304.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200302
ENTRY DATE: Entered STN: 31 Dec 2002
Last Updated on STN: 14 Feb 2003
Entered Medline: 13 Feb 2003

AB In the kidney, 25-hydroxyvitamin D(3) (25(OH)D) is converted to 1,25-dihydroxyvitamin D(3) (1,25(OH)(2)D) by the 25(OH)D(3)-1alpha-hydroxylase enzyme, which contains a terminal cytochrome P450 (CYP1alpha) (systematic name: CYP27B1). Likewise, the kidney also produces 24,25-dihydroxyvitamin D(3) and 1,24,25-trihydroxyvitamin D(3) via a 24-hydroxylase whose terminal cytochrome P450 is CYP24. The purpose of this study was to characterize the transcriptional regulation of the CYP1alpha and CYP24 genes by parathyroid hormone (PTH) and 1,25(OH)(2)D in the kidney. Promoter-reporter gene constructs were transfected into opossum kidney (OK) cells, a renal proximal tubular cell line with endogenous PTH and 1,25(OH)(2)D receptors. PTH and forskolin stimulated CYP1alpha promoter activity via a cAMP-dependent pathway acting through the phosphorylation of CREB (cAMP-dependent response element-binding protein). This stimulation did not require new protein synthesis but may be modulated by short-lived proteins. 1,25(OH)(2)D modestly inhibited basal and forskolin-stimulated CYP1alpha promoter activity. The stimulation of

CYP1alpha promoter activity by PTH and forskolin can account for the effect of these hormones on renal CYP1alpha mRNA levels. CYP24 promoter activity in transfected cells was increased by both 1,25(OH)(2)D and PTH, but there was no interaction between the two. The modest effects of 1,25(OH)(2)D and PTH on promoter activity and their lack of interaction do not account for the effects of these hormones on renal CYP24 mRNA levels. This suggests that there may be important posttranscriptional regulation of CYP24 mRNA in the kidney.

L14 ANSWER 12 OF 21 MEDLINE on STN
ACCESSION NUMBER: 2002734753 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12498308
TITLE: Development of a vitamin D-responsive organ culture system for adult and old rat intestine.
AUTHOR: Armbrecht H J; Boltz M A; Kumar V B
CORPORATE SOURCE: Geriatric Research, Education, and Clinical Center, St. Louis VA Medical Center, St. Louis, Missouri 63125, USA.
CONTRACT NUMBER: AG-12587 (NIA)
SOURCE: Digestive diseases and sciences, (2002 Dec) Vol. 47, No. 12, pp. 2831-8.
Journal code: 7902782. ISSN: 0163-2116.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200301
ENTRY DATE: Entered STN: 27 Dec 2002
Last Updated on STN: 11 Jan 2003
Entered Medline: 10 Jan 2003

AB The purpose of this study was to develop an organ culture system for adult and old rat small intestine that is responsive to vitamin D. Explants from F344 rats were cultured on Millipore tissue culture inserts placed in 6-well dishes at a temperature of 28 degrees C and in the presence of 95% oxygen. Explants from young (2 months old), adult (12 months old), and old (22 months old) rats were viable for up to 12 hr as determined by constant rates of DNA and protein synthesis. Hormonal responsiveness was characterized by measuring the capacity of 1,25-dihydroxyvitamin D [1,25(OH)2D], the hormonal form of vitamin D, to increase mRNA levels of the intestinal 24-hydroxylase cytochrome P-450 (CYP24). Jejunal explants from young rats increased CYP24 mRNA levels in a linear fashion with an EC50 of 3 nM in response to 1,25(OH)2D. There was no change with age in the magnitude of the jejunal response with regard to time (0-12 hr) or dose (0.1-100 nM). However, in the duodenum, 1,25(OH)2D increased CYP24 mRNA to significantly higher levels in the adult compared to the young. Since the 24-hydroxylase is the first step in the degradative pathway for 1,25(OH)2D in the intestine, increased duodenal expression of the 24-hydroxylase may contribute to the decreased action of 1,25(OH)2D on the adult duodenum.

L14 ANSWER 13 OF 21 MEDLINE on STN
ACCESSION NUMBER: 2001611802 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11686044
TITLE: Enzymatic studies on the key enzymes of vitamin D metabolism; 1 alpha-hydroxylase (CYP27B1) and 24-hydroxylase (CYP24).
AUTHOR: Inouye K; Sakaki T
CORPORATE SOURCE: Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502,

Japan.. inouye@kais.kyoto-u.ac.jp
SOURCE: Biotechnology annual review, (2001) Vol. 7, pp. 179-94.
Ref: 40
Journal code: 9616443. ISSN: 1387-2656.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200204
ENTRY DATE: Entered STN: 5 Nov 2001
Last Updated on STN: 9 Apr 2002
Entered Medline: 8 Apr 2002

AB The key enzymes of vitamin D3 metabolism, renal 25-hydroxyvitamin D3 1 alpha-hydroxylase (CYP27B1) and 24-hydroxylase (CYP24) were expressed in Escherichia coli, and their enzymatic properties were revealed. As expected, mouse CYP27B1 and human CYP27B1 showed the 1 alpha-hydroxylation of 25-hydroxyvitamin D3 with the Michaelis constant, Km, value of 2.7 microM. Unexpectedly, both mouse CYP27B1 and human CYP27B1 showed greater Vmax/Km values toward 24,25-dihydroxyvitamin D3 than 25-hydroxyvitamin D3, suggesting that 24, 25-dihydroxyvitamin D3 is a better substrate than 25-hydroxyvitamin D3 for both CYP27B1. Enzymatic studies on substrate specificity of CYP27B1 revealed that 25-hydroxyl group of vitamin D3 was essential for the 1 alpha-hydroxylase activity, and 24-hydroxyl group enhanced the activity, but, 23-hydroxyl group greatly reduced the activity. On rat CYP24, it was demonstrated that CYP24 catalyzed four-step monooxygenation towards 25-hydroxyvitamin D3. Furthermore, in vivo and in vitro metabolic studies on 1 alpha,25-dihydroxyvitamin D3 clearly indicated that CYP24 catalyzed six-step monooxygenation to convert 1 alpha,25-dihydroxyvitamin D3 into calcitroic acid which is known as a final metabolite of 1 alpha,25-dihydroxyvitamin D3 for excretion in bile. These results strongly suggest that CYP24 is highly responsible for the metabolism of both 25-hydroxyvitamin D3 and 1 alpha,25-dihydroxyvitamin D3. In addition, we have succeeded in the construction of mitochondrial P450 electron transport chain consisting of ADR, ADX and each of CYP27B1 and CYP24 in E. coli cells. The coexpression system with CYP27B1 might be useful as a bioreactor to produce 1 alpha,25-dihydroxyvitamin D3. In contrast, the coexpression system with CYP24 would be applied to metabolic studies of vitamin D analogs used as drugs.

L14 ANSWER 14 OF 21 MEDLINE on STN
ACCESSION NUMBER: 2001227704 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11250655
TITLE: Differential responsiveness of intestinal epithelial cells to 1,25-dihydroxyvitamin D3--role of protein kinase C.
AUTHOR: Armbrecht H J; Boltz M A; Hodam T L; Kumar V B
CORPORATE SOURCE: Geriatric Research, Education, and Clinical Center, St Louis VA Medical Center, St Louis, Missouri 63125, USA.
CONTRACT NUMBER: AG-12587 (NIA)
SOURCE: The Journal of endocrinology, (2001 Apr) Vol. 169, No. 1, pp. 145-51.
Journal code: 0375363. ISSN: 0022-0795.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200104

ENTRY DATE: Entered STN: 2 May 2001
Last Updated on STN: 2 May 2001
Entered Medline: 26 Apr 2001

AB Non-transformed rat intestinal epithelial cell (IEC) lines were used to study the action of 1,25-dihydroxyvitamin D(3) (1,25(OH)2D) in the intestine. The capacity of 1,25(OH)2D to increase the expression of the cytochrome P450 component of the vitamin D 24 -hydroxylase (CYP24) was determined in IEC-6 and IEC-18 cell lines. In IEC-6 cells, which are derived from crypt cells isolated from the whole small intestine, 1,25(OH)2D markedly increased expression of CYP24 protein and mRNA within 12 h. In contrast, in IEC-18 cells, which are derived from crypt cells from the ileum only, 1,25(OH)2D did not increase expression of CYP24 until 24-48 h. The maximal levels of CYP24 mRNA seen in the IEC-18 cells were only 31% of the maximal levels seen in the IEC-6 cells. In the presence of 1,25(OH)2D, phorbol esters rapidly increased CYP24 mRNA levels in IEC-18 cells from almost undetectable to levels seen in IEC-6 cells. Protein kinase inhibitors abolished the stimulation by 1,25(OH)2D and by phorbol esters in both cell lines. Stimulation of mRNA levels by phorbol esters required new protein synthesis but stimulation by 1,25(OH)2D did not. These studies demonstrated that the rapid action of 1,25(OH)2D in IEC-6 cells is related to the activation of protein kinase C, an event which is missing in the IEC-18 cells. This differential response to 1,25(OH)2D probably takes place at a post-receptor site, since the number of vitamin D receptors in each cell line was found to be similar.

L14 ANSWER 15 OF 21 MEDLINE on STN
ACCESSION NUMBER: 2001037861 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11012668
TITLE: Dual metabolic pathway of 25-hydroxyvitamin D3 catalyzed by human CYP24.
AUTHOR: Sakaki T; Sawada N; Komai K; Shiozawa S; Yamada S; Yamamoto K; Ohyama Y; Inouye K
CORPORATE SOURCE: Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Japan.
SOURCE: European journal of biochemistry / FEBS, (2000 Oct) Vol. 267, No. 20, pp. 6158-65.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200011
ENTRY DATE: Entered STN: 22 Mar 2001
Last Updated on STN: 22 Mar 2001
Entered Medline: 28 Nov 2000

AB Human 25-hydroxyvitamin D3 (25(OH)D3) 24-hydroxylase (CYP24) cDNA was expressed in Escherichia coli, and its enzymatic and spectral properties were revealed. The reconstituted system containing the membrane fraction prepared from recombinant E. coli cells, adrenodoxin and adrenodoxin reductase was examined for the metabolism of 25(OH)D3, 1alpha,25(OH)2D3 and their related compounds. Human CYP24 demonstrated a remarkable metabolism consisting of both C-23 and C-24 hydroxylation pathways towards both 25(OH)D3 and 1alpha,25(OH)2D3, whereas rat CYP24 showed almost no C-23 hydroxylation pathway [Sakaki, T. Sawada, N. Nonaka, Y. Ohyama, Y. & Inouye, K. (1999) Eur. J. Biochem. 262, 43-48]. HPLC analysis and mass spectrometric analysis revealed that human CYP24 catalyzed all

the steps of the C-23 hydroxylation pathway from 25(OH)D3 via 23S, 25(OH)2D3, 23S,25,26(OH)3D3 and 25(OH)D3-26,23-lactol to 25(OH)D3-26, 23-lactone in addition to the C-24 hydroxylation pathway from 25(OH)D3 via 24R,25(OH)2D3, 24-oxo-25(OH)D3, 24-oxo-23S,25(OH)2D3 to 24,25,26,27-tetranor-23(OH)D3. On 1alpha,25(OH)2D3 metabolism, similar results were observed. These results strongly suggest that the single enzyme human CYP24 is greatly responsible for the metabolism of both 25(OH)D3 and 1alpha,25(OH)2D3. We also succeeded in the coexpression of CYP24, adrenodoxin and NADPH-adrenodoxin reductase in E. coli. Addition of 25(OH)D3 to the recombinant E. coli cell culture yielded most of the metabolites in both the C-23 and C-24 hydroxylation pathways. Thus, the E. coli expression system for human CYP24 appears quite useful in predicting the metabolism of vitamin D analogs used as drugs.

L14 ANSWER 16 OF 21 MEDLINE on STN
ACCESSION NUMBER: 1999248063 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10231362
TITLE: Metabolic studies using recombinant escherichia coli cells producing rat mitochondrial CYP24 CYP24 can convert 1alpha,25-dihydroxyvitamin D3 to calcitroic acid.
AUTHOR: Sakaki T; Sawada N; Nonaka Y; Ohyama Y; Inouye K
CORPORATE SOURCE: Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Japan.
SOURCE: European journal of biochemistry / FEBS, (1999 May) Vol. 262, No. 1, pp. 43-8.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 12 Jul 1999
Last Updated on STN: 12 Jul 1999
Entered Medline: 21 Jun 1999
AB Previously we expressed rat 25-hydroxyvitamin D3 24-hydroxylase (CYP24) cDNA in Escherichia coli JM109 and showed that CYP24 catalyses three-step monooxygenation towards 25-hydroxyvitamin D3 and 1alpha,25-dihydroxyvitamin D3 [Akiyoshi-Shibata, M., Sakaki, T., Ohyama, Y., Noshiro, M., Okuda, K. & Yabusaki, Y. (1994) Eur. J. Biochem. 224, 335-343]. In this study, we demonstrate further oxidation by CYP24 including four- and six-step monooxygenation towards 25-hydroxyvitamin D3 and 1alpha,25-dihydroxyvitamin D3, respectively. When the substrate 25-hydroxyvitamin D3 was added to a culture of recombinant E. coli, four metabolites, 24, 25-dihydroxyvitamin D3, 24-oxo-25-hydroxyvitamin D3, 24-oxo-23, 25-dihydroxyvitamin D3 and 24,25,26,27-tetranor-23-hydroxyvitamin D3 were observed. These results indicate that CYP24 catalyses at least four-step monooxygenation toward 25-hydroxyvitamin D3. Furthermore, in-vivo and in-vitro metabolic studies on 1alpha,25-dihydroxyvitamin D3 clearly indicated that CYP24 catalyses six-step monooxygenation to convert 1alpha,25-dihydroxyvitamin D3 into calcitroic acid which is known as a final metabolite of 1alpha,25-dihydroxyvitamin D3 for excretion in bile. These results strongly suggest that CYP24 is largely responsible for the metabolism of both 25-hydroxyvitamin D3 and 1alpha,25-dihydroxyvitamin D3.

L14 ANSWER 17 OF 21 MEDLINE on STN
ACCESSION NUMBER: 1999011334 MEDLINE

DOCUMENT NUMBER: PubMed ID: 9792969
TITLE: Effect of 26,26,26,27,27,27-Hexafluoro-1,25-dihydroxyvitamin D3 on the expression of vitamin-D-responsive genes in vitamin-D-deficient mice.
AUTHOR: Yoshimura T; Itoh S; Tsujikawa K; Yamada E; Ishii T; Iemura O; Kameda Y; Mimura T; Kohama Y
CORPORATE SOURCE: Faculty of Pharmaceutical Sciences, Osaka University, Osaka, Japan.
SOURCE: Pharmacology, (1998 Dec) Vol. 57, No. 6, pp. 286-94.
Journal code: 0152016. ISSN: 0031-7012.
PUB. COUNTRY: Switzerland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 15 Jan 1999
Last Updated on STN: 15 Jan 1999
Entered Medline: 4 Jan 1999

AB 26,26,26,27,27,27-Hexafluoro-1,25-dihydroxyvitamin D3 (ST-630) is a newly developed agent to maintain the levels of calcium and phosphorus in blood. Herein, we investigated the effect of this compound on the expression of vitamin-D-responsive genes in vitamin-D-deficient mice. ST-630 was more effective than 1, 25-dihydroxyvitamin D3 [1,25(OH)2D3] with respect to the induction of Cyp24 and calbindin-D9k mRNAs in the kidney and in the small intestine. Moreover, the increase in mRNA levels of vitamin-D-responsive genes induced by ST-630 lasted longer than that induced by 1,25(OH)2D3. These results indicate that ST-630 was more effective in inducing Cyp24 and calbindin-D9k gene expression than 1, 25(OH)2D3 when both compounds were injected into vitamin-D-deficient mice.

L14 ANSWER 18 OF 21 MEDLINE on STN
ACCESSION NUMBER: 1998349944 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9687155
TITLE: Repression of basal transcription by vitamin D receptor: evidence for interaction of unliganded vitamin D receptor with two receptor interaction domains in RIP13deltal.
AUTHOR: Dwivedi P P; Muscat G E; Bailey P J; Omdahl J L; May B K
CORPORATE SOURCE: Department of Biochemistry, University of Adelaide, South Australia, Australia.
SOURCE: Journal of molecular endocrinology, (1998 Jun) Vol. 20, No. 3, pp. 327-35.
Journal code: 8902617. ISSN: 0952-5041.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 20 Oct 1998
Last Updated on STN: 20 Oct 1998
Entered Medline: 5 Oct 1998

AB Repression of basal transcription of a 1,25-dihydroxyvitamin D3 (1,25-(OH)2D3) responsive 25-hydroxyvitamin D3-24-hydroxylase (CYP24) promoter construct as observed in kidney cells in the absence of ligand and this repression was dependent on a functional vitamin D response element (VDRE). Basal repression was also seen with a construct where a consensus DR-3-type VDRE was fused to the thymidine kinase promoter. Expression of a dominant negative vitamin D receptor (VDR) isoform that strongly bound to the VDRE motif in the CYP24 promoter ablated basal repression. This VDR isoform lacked sequence in

the hinge- and ligand-binding domains implicating one or both of these domains in basal repression. It is well known that thyroid hormone and retinoic acid receptors silence basal transcription of target genes in the absence of ligands and this repressor function can be mediated by the nuclear receptor corepressor N-CoR. Two variants of N-CoR have been described, RIP13a and RIP13delta1. N-CoR and the variants contain two receptor interaction domains, ID-I and ID-II, which are identical except region ID-II in RIP13delta1 has an internal deletion. We have used the mammalian two hybrid system to investigate whether VDR, in the absence of ligand 1,25-(OH)2D3, can interact with these domains. The data showed that unliganded VDR does not interact with either ID-I or ID-II from RIP13a and RIP13delta1, but does interact strongly with a composite domain of ID-I and ID-II from RIP13delta1 (but not from RIP13a) and this strong interaction is abrogated in the presence of ligand. This finding implicates RIP13delta1 in VDR-dependent basal repression of the promoter constructs under investigation. However, over-expression of RIP13delta1 in kidney cell lines did not alter basal expression of the CYP24 promoter construct. It is concluded that either the level of endogenous RIP13delta1 in these kidney cells permits maximal repression or that repression occurs by a mechanism that is independent of RIP13delta1. Alternatively, repression may be dependent on RIP13delta1 but requires an additional cofactor that is limiting in these cells.

L14 ANSWER 19 OF 21 MEDLINE on STN
ACCESSION NUMBER: 1998344810 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9681485
TITLE: Induction of the vitamin D 24 -hydroxylase (CYP24) by 1,25-dihydroxyvitamin D3 is regulated by parathyroid hormone in UMR106 osteoblastic cells.
AUTHOR: Armbrecht H J; Hodam T L; Boltz M A; Partridge N C; Brown A J; Kumar V B
CORPORATE SOURCE: Geriatric Research, Education and Clinical Center, St. Louis VA Medical Center, Missouri 63125, USA.
CONTRACT NUMBER: AG-12587 (NIA)
SOURCE: Endocrinology, (1998 Aug) Vol. 139, No. 8, pp. 3375-81. Journal code: 0375040. ISSN: 0013-7227.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Space Life Sciences
ENTRY MONTH: 199808
ENTRY DATE: Entered STN: 20 Aug 1998
Last Updated on STN: 24 Jan 2002
Entered Medline: 13 Aug 1998

AB The expression of the vitamin D 24-hydroxylase is highly regulated in target tissues for 1,25-dihydroxyvitamin D3 (1,25(OH)2D), where it may modulate the action of 1,25(OH)2D. In UMR106 osteoblastic cells, 1,25(OH)2D and PTH synergistically induce 24-hydroxylase expression. The purpose of these studies was to characterize the interaction between 1,25(OH)2D and PTH with regard to the messenger RNA (mRNA) levels of the cytochrome P450 component of the 24-hydroxylase (CYP24). PTH alone had no effect on CYP24 mRNA levels, and 1,25(OH)2D alone produced only a modest increase. However, 1,25(OH)2D and PTH together synergistically increased CYP24 mRNA levels 3-fold compared with 1,25(OH)2D alone. PTH also increased the sensitivity of UMR cells to 1,25(OH)2D from 10(-8) to 10(-10) M. PTH worked through the cAMP signaling pathway as evidenced by the lack of effect of PTH (3-34) and by

the full activity of 8-bromo-cAMP. PTH in the presence of 1,25(OH)2D increased CYP24 gene transcription as shown by nuclear run-on studies and by activation of a CYP24 promoter-reporter construct after transfection. PTH also increased vitamin D receptor number in UMR cells, but this occurred at times later than the increase in transcription. These studies demonstrate that PTH in the presence of 1,25(OH)2D works through the cAMP-dependent signaling pathway to increase transcription of the CYP24 gene, to increase CYP24 protein levels, and to increase 24-hydroxylase activity.

L14 ANSWER 20 OF 21 MEDLINE on STN
ACCESSION NUMBER: 1998286051 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9622848
TITLE: Rat CYP24 catalyses 23S-hydroxylation of 26,26,26,27,27,27-hexafluorocalcitriol in vitro.
AUTHOR: Hayashi K; Akiyoshi-Shibata M; Sakaki T; Yabusaki Y
CORPORATE SOURCE: Biotechnology Laboratory, Sumitomo Chemical Co., Ltd, Hyogo, Japan.
SOURCE: Xenobiotica; the fate of foreign compounds in biological systems, (1998 May) Vol. 28, No. 5, pp. 457-63. Journal code: 1306665. ISSN: 0049-8254.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199808
ENTRY DATE: Entered STN: 28 Aug 1998
Last Updated on STN: 28 Aug 1998
Entered Medline: 20 Aug 1998

AB 1. Kidney mitochondrial 24-hydroxylase cytochrome P450 (CYP24) catalyses sequential hydroxylation at both C-24 and C-23 positions of calcidiol and calcitriol. Here, we have investigated the in vitro metabolism of a hexafluorinated derivative of calcitriol, 26,26,26,27,27,27-hexafluorocalcitriol (ST-630), in a reconstituted system by using recombinant Escherichia coli membrane fractions containing rat CYP24. 2. When ST-630 was incubated with CYP24 supplemented with bovine adrenodoxin and NADPH-adrenodoxin reductase, a distinct metabolite could be observed. This metabolite was found to be 26,26,26,27,27,27-hexafluoro-23S-hydroxcalcitriol, a biologically active metabolite of ST-630, based on cochromatography on HPLC and mass spectrometric analysis. 3. These results show the direct evidence that CYP24 plays an essential role in the metabolism of ST-630 to yield its 23S-hydroxylated metabolite, as observed in cultured cells and experimental animal studies.

L14 ANSWER 21 OF 21 MEDLINE on STN
ACCESSION NUMBER: 96397534 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8804428
TITLE: Insulin markedly potentiates the capacity of parathyroid hormone to increase expression of 25-hydroxyvitamin D3-24-hydroxylase in rat osteoblastic cells in the presence of 1,25-dihydroxyvitamin D3.
AUTHOR: Armbrecht H J; Wongsurawat V J; Hodam T L; Wongsurawat N
CORPORATE SOURCE: Geriatric Research, Education and Clinical Center, Veterans Affairs Medical Center, St. Louis, MO 63125, USA.
CONTRACT NUMBER: AG 12587 (NIA)
SOURCE: FEBS letters, (1996 Sep 9) Vol. 393, No. 1, pp. 77-80. Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19 Dec 1996
Last Updated on STN: 19 Dec 1996
Entered Medline: 7 Nov 1996

AB We have previously shown that insulin alters the renal metabolism of 25-hydroxyvitamin D. To examine the effect of insulin on vitamin D metabolism in bone, we have used UMR-106 osteoblast-like cells to study the regulation of 25(OH)D3-24-hydroxylase (24-hydroxylase) expression by insulin. The 24-hydroxylase is an important enzyme in degrading 1,25-dihydroxyvitamin D3 (1,25(OH)2D) in target tissues. Insulin alone had no effect on mRNA levels of the cytochrome P450 component (CYP24) of the 24-hydroxylase or on 24-hydroxylase activity itself in UMR cells. However, insulin increased the capacity of parathyroid hormone (PTH) to elevate CYP24 mRNA levels by 3-4-fold and to increase 24-hydroxylase activity by 2-fold in the presence of 1,25(OH)2D. Insulin increased the maximal responsiveness of UMR cells to PTH without altering their sensitivity. The action of insulin required the presence of 1,25(OH)2D and was partly dependent on new protein synthesis. Insulin-like growth factor 1 also potentiated the effects of PTH. This marked stimulation of the 24-hydroxylase by PTH and insulin may serve to regulate 1,25(OH)2D action and/or to produce 24,25-dihydroxyvitamin D in bone cells.

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=> => d que stat l15
L1             2 SEA FILE=REGISTRY ABB=ON  ("VITAMIN D 24-HYDROXYLASE (MOUSE
                 PRECURSOR)"/CN OR "VITAMIN D 25-HYDROXYLASE"/CN)
L2           302 SEA FILE=HCAPLUS ABB=ON  L1 OR ?VITAMIN?(W)D(W)24(W)?HYDROXYLAS
                 E?
L3            26 SEA FILE=HCAPLUS ABB=ON  L2 AND CYP24
L4            10 SEA FILE=HCAPLUS ABB=ON  L3 AND MRNA
L5            26 SEA FILE=HCAPLUS ABB=ON  L3 OR L4
L6             8 SEA FILE=HCAPLUS ABB=ON  L5 AND ?PROTEIN?
L7            26 SEA FILE=HCAPLUS ABB=ON  L5 OR L6
L8             9 SEA FILE=HCAPLUS ABB=ON  L7 AND (?DETECT? OR ?IDENT? OR
                 ?ISOLAT?)
L9            26 SEA FILE=HCAPLUS ABB=ON  L7 OR L8
L15            1 SEA FILE=USPATFULL ABB=ON  L9 AND (PRD<19990402 OR PD<19990402)

=> d ibib abs l15 1

L15  ANSWER 1 OF 1  USPATFULL on STN
ACCESSION NUMBER:        2004:221287  USPATFULL
TITLE:                   Gene sequence variations with utility in determining
                         the treatment of disease, in genes relating to drug
                         processing
INVENTOR(S):             Stanton, Vincent P., JR., Belmont, MA, UNITED STATES
PATENT ASSIGNEE(S):      Variagenics, Inc., a Delaware corporation (U.S.
                         corporation)

                              NUMBER        KIND    DATE
                         ---------------- ------ --------
PATENT INFORMATION:      US 2004171056      A1   20040902
APPLICATION INFO.:       US 2004-798873     A1   20040311  (10)
RELATED APPLN. INFO.:    Continuation of Ser. No. US 2000-648123, filed on 25
                         Aug 2000, ABANDONED Continuation-in-part of Ser. No. US
                         2000-590783, filed on 8 Jun 2000, ABANDONED
                         Continuation-in-part of Ser. No. US 2000-501955, filed
                         on 10 Feb 2000, ABANDONED Continuation-in-part of Ser.
                         No. WO 2000-US1392, filed on 20 Jan 2000, PENDING
                         Continuation-in-part of Ser. No. US 1999-451252, filed
                         on 29 Nov 1999, ABANDONED Continuation-in-part of Ser.
                         No. US 1999-427835, filed on 26 Oct 1999, ABANDONED
                         Continuation-in-part of Ser. No. US 1999-414330, filed
                         on 6 Oct 1999, ABANDONED Continuation-in-part of Ser.
                         No. US 1999-389993, filed on 3 Sep 1999, ABANDONED
                         Continuation-in-part of Ser. No. US 1999-370841, filed
                         on 9 Aug 1999, ABANDONED Continuation-in-part of Ser.
                         No. US 1999-300747, filed on 26 Apr 1999, ABANDONED

                                NUMBER         DATE
                         ------------------ --------
PRIORITY INFORMATION:    US 1999-121047P    19990222 (60)           <--
                         US 1999-131334P    19990426 (60)
                         US 1999-131191P    19990426 (60)
                         US 1999-139440P    19990615 (60)
DOCUMENT TYPE:           Utility
FILE SEGMENT:            APPLICATION
LEGAL REPRESENTATIVE:    FISH & RICHARDSON PC, 225 FRANKLIN ST, BOSTON, MA,
                         02110
NUMBER OF CLAIMS:        16
EXEMPLARY CLAIM:         1
LINE COUNT:              11893
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods for identifying and utilizing variances in genes relating to efficacy and safety of medical therapy and other aspects of medical therapy are described, including methods for selecting an effective treatment.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.